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## THE MALE GAMETOPHYTE OF DACRYDIUM

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY, 99

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(WITH PLATE XIX)

Until 1902, when COKER (1) published a paper on the gametophytes and embryo of *Podocarpus*, we had no account whatever of any of the Podocarpineae. The fact that they are in the main forms of the southern hemisphere has made it hard to secure the necessary material. COKER's study was made from two cultivated trees, and his results, so far as the male gametophyte is concerned, were, very briefly, as follows: (1) The pollen grain contains two prothallial cells; the first degenerates slowly, the nucleus of the second slips out into the general cytoplasm and may divide; the first may segment. (2) The mature pollen grain sometimes contains as many as six nuclei. (3) There is one functional male cell formed.

In connection with these results it is interesting to note some recent work on Araucarieae. In 1905 THOMPSON (2) found in the pollen tubes of *Agathis* supernumerary nuclei, sometimes as many as thirteen, but was not certain of their origin. In the same year he found a similar condition in *Araucaria*, and in one instance at least, there were more than thirty nuclei placed fore and aft. In 1905 LOPRIORE (4) described the pollen grains of *Araucaria Bidwillii*. According to his account, two lenticular cells are successively cut off from the main body of the spore, and from them a complex is developed. The free central nucleus and an inner cell of the complex increase in size and undergo no further divisions; they remain distinct from the others, and even in the germinating tube can be recognized. One of them is figured with a conspicuous envelope of cytoplasm rich in starch. The complex increases to about fifteen cells when the walls disappear. Nuclear division, however, continues until there are about thirty-six free nuclei in the general cytoplasm. LOPRIORE interprets the cell complex as representing an antheridium and considers the two large nuclei as vegetative in character. The paper has been reviewed by

CHAMBERLAIN (5), who suggests that the complex is prothallial and the two nuclei reproductive.

In 1904 JUEL (6) found in the pollen tubes of *Cupressus Goveniana* a complex of male cells; sometimes there were only four, but more often eight or ten, and in one case about twenty. The tree was grown in a greenhouse and may not have been normal. Recently, however, a study of *Microcycas calocoma* by CALDWELL (7) has revealed a somewhat similar multiplication of reproductive cells. He found in the pollen tube a tube nucleus, a prothallial cell, a stalk cell, and eight or nine body cells. Each of these body cells later divides to form two sperm mother cells.

The peculiarity of the pollen grains of Podocarpus and its possible bearing on the question of relationships among conifers added considerable interest to the study of *Dacrydium*. A brief summary of some points in our present knowledge of the male gametophytes of conifers may be useful.

1. *Prothallial cells*.—Taxineae, Taxodineae, and Cupressineae have none. In Abietineae there are two and both are ephemeral. In Podocarpus there are originally two; one at least is persistent and may divide. In Araucaria, as CHAMBERLAIN interprets LOPRIORE's figures, there is extensive prothallial tissue.

2. *Generative cells*.—The free nucleus of the spore divides to form the generative cell and tube nucleus. Usually the generative cell divides periclinally to form the so-called stalk and body cells, of which the former is sterile.

3. *The male cells*.—The body cell divides to form two male cells or nuclei. The sperms are usually unequal, but in *Taxodium* and *Juniperus*, and perhaps in *Pinus*, they are equal. A complex of male cells has been found in *Cupressus Goveniana*.

4. *Shedding*.—The usual shedding-stage is after the division into tube nucleus and generative cell; but in *Juniperus* and *Cupressus* the microspores are shed before any division has taken place; and in *Picea* the generative cell has divided.

#### DACRYDIUM

The material for the present work was collected by Professor L. COCKAYNE in December 1906 and January 1907 in New Zealand.

It was killed and preserved in 70 per cent. alcohol and formalin, imbedded in paraffin, cut 5  $\mu$ , and stained part in 0.5 per cent. hematoxylin and orange G and part in safranin and gentian violet. The material consisted of staminate strobili of *Dacrydium biforme*, *D. Bidwillii*, *D. cupressinum*, and *D. laxifolium*, and young ovules of *D. cupressinum*, *D. laxifolium*, and *D. intermedium*. Unfortunately, it was impossible to obtain a complete series from any one species. The first named was in the microspore stage only; the second furnished a series from the microspore up to the shedding of the pollen; the pollen of the third was ready to be shed, but no grains were found in the ovules; the fourth showed young tubes in the micropyles, and in *D. intermedium* they had penetrated the nucellus.

The staminate strobili are 2 to 6<sup>mm</sup> in length and terminate short branches. The nucleus of the microspore is large and lies near the base of the spore, that is, the side opposite the wings (fig. 1). The first division cuts off a lenticular prothallial cell which lies close against the basal wall (fig. 2). Another division soon follows, cutting off from the main body of the spore a second prothallial cell, similar to the first and overlying it (figs. 3, 4). Both these cells have distinct though very delicate walls; their nuclei are somewhat flattened and stain very deeply, but they show no sign of degeneration; the cytoplasm also is very dense.

In *Dacrydium*, as in *Podocarpus* and the Abietineae, a third cell is now cut off from the main body of the spore (fig. 5). It overlies the others and is so similar to them that but for its subsequent behavior one might think it a third prothallial cell. It is a generative cell—generative in the sense that it is the ancestor of sperms. This and the second prothallial cell now both divide; usually the division of the latter occurs first, but the order may be reversed (figs. 6, 7). In either case the result is a complex of five distinct cells, one in the lowest, and two in each of the upper tiers, filling about one-third of the cavity of the spore. All the five nuclei are oval and stain very deeply. The free nucleus, which is now the tube nucleus, divides no more. It is somewhat irregular in outline and is larger and less dense than any of the others. The cytoplasm of the pollen grains is at this time considerably vacuolated.

Up to this point all the stages described were obtained from *D.*

*Bidwillii*. A very close series was secured, showing spindles in every one of the divisions, so that the sequence of events was quite clear. In this species, very rarely, a division of the first prothallial cell occurs. In *D. cypressinum*, however, this takes place normally, giving a complex of six instead of five cells (*fig. 11*). As early stages were not secured, I cannot say when the division occurs.

The next step in the development of the gametophyte is the enlargement of the two generative daughter cells. They increase in thickness and the nuclei lose their flattened appearance. Before this there had been nothing by which to distinguish between the two, but now one of them grows more rapidly than the other and arches upward conspicuously. This may take place before or after the division of the second prothallial cell. At the same time the upper and inner walls become gradually less and less distinct and finally disappear altogether (*figs. 8, 9, 10*). The larger cell does not lose its identity, but retains about itself a sheath of cytoplasm, rich in starch. It abandons all connection with the spore wall and appears as a rounded cell free within the pollen grain, similar to the body cell of other conifers. The other loses itself in the general cytoplasm and its nucleus is set free.

The prothallial cells now also increase somewhat in thickness, their nuclei round up, and their walls fade away. When ready to be shed, the pollen grain of *D. Bidwillii* contains the body cell and five free nuclei. These change their relative positions somewhat, and the nucleus of the body cell is indistinguishable from those of the prothallial cells and the tube nucleus.

Several pollen grains of *D. Bidwillii* were found in which two body cells were apparently developing from the divided generative cell; though these were unequal in size, both had the characteristic sheath. In two or three older pollen grains of *D. laxifolium*, in which the tubes were formed, there was the appearance of two body cells, one larger than the other; *fig. 13* shows one of these cases, though not the most pronounced.

*D. laxifolium*, though collected on the same day with the microspores of *D. bifforme* and the more advanced *D. Bidwillii*, showed no young stages at all. The pollen had all been shed. The micropyles were full of the pollen grains, of which some had not germinated,

but some had tubes twice the length of the spore. In these there appeared always the conspicuous body cell and six free nuclei. Among the latter one could sometimes recognize the tube and generative daughter nuclei by their position or appearance, but sometimes they were quite indistinguishable. In several cases one, presumably the tube nucleus, had entered the tube and the others were about to follow it (*figs. 12, 13*).

From the number of free nuclei, it is fair to infer that in *D. laxifolium*, as in *D. cypressinum*, the first prothallial cell has divided. Another possible explanation would be the division of the body cell, but the latest stages in *D. Bidwillii*, in which the shedding of the pollen had begun, could not have been much earlier than that of the ungerminated pollen grains in the micropyles, and there was no evidence of such a division in either of these. In several cases, too, the position of two free nuclei side by side against the lower wall suggested their origin from the first prothallial cell.

The latest stages found were in *D. intermedium* collected January 31. The tubes had penetrated the nucellus to some distance, and free nuclei were seen in them, but there was no evidence of any further divisions. The body cell, still undivided, remained alone in the cavity of the pollen grain. It seemed probable that it would have divided and given rise directly to two sperms, as in other conifers, but there might be other divisions previous to sperm formation. It is hoped that in the near future material can be secured and the life-history completed.

#### DISCUSSION

The striking facts about the male gametophyte are (1) the multiplication of prothallial cells and (2) the transverse division of the generative cell. The development in *Dacrydium* agrees rather closely with that described by COKER for *Podocarpus*, except that there is no sign of degeneration of any of the cells, and the division of the generative cell in *Podocarpus* was not observed. In the light of what we now know about *Podocarpineae*, one can hardly study LOPRIORE's drawings of *Araucaria* without agreeing with CHAMBERLAIN'S view. The mode of origin of the cell complex at once suggests the prothallial cells of other conifers, and the large nucleus with its cytoplasmic sheath looks too much like a body cell to be called vegeta-

tive. In fact, all the early figures up to the five-cell stage might be taken to represent *Dacrydium cupressinum*. These show the formation of first and second prothallial cells, the generative cell, and the division of the first prothallial cell. According to LOPRIORE's account, however, after the first two divisions it is usually the middle, that is the second prothallial cell, which divides longitudinally, cutting off what we would call the generative cell.

The complex in *Cupressus* is not at all comparable to that of Araucaria, for it has a wholly different origin. It is not formed early in the history of the gametophyte by successive divisions of the spore, but later, in the tube, by the division of a single differentiated reproductive or body cell. The rarity of any prothallial cells at all in conifers sets apart Abietineae, Podocarpineae, and Araucarineae as the possible representatives of a more primitive condition. Araucaria seems to point toward an ancestral functioning thallus, and *Dacrydium* shows a further step in the reduction so nearly accomplished in *Pinus*. A possible connection between the three groups is suggested. The question of relationships, however, can be answered only through the combined results of various lines of research.

In most conifers that have been studied, the generative cell divides periclinally to form the so-called stalk and body cells, but outside of conifers we find *Cycas* and *Ginkgo* in which the division is transverse. The term "stalk cell" is a rather unfortunate one. Its use was suggested by the position of the cell between the body and the prothallial cells, but in the case of a transverse division, or where, as in *Sequoia* and *Cryptomeria*, the division takes place in the free condition in the tube, the significance of the name is entirely lost. It is perhaps misleading, too, in the suggestion of the stalk of an antheridium.

The generative cell is the first of a spermatogenous series. In *Pinus*, for example, it divides, giving rise to a sterile and a fertile cell. The body cell divides once and produces two male cells. In *Dacrydium* also the first division gives rise to a fertile and a sterile cell, but with a tendency for both to function. In *Microcycas* the generative cell produces the sterile stalk cell and eight or more body cells. In *Cupressus Goveniana* one at least of the two generative daughter cells divides many times, giving rise to extensive fertile tissue. JUEL

saw a small nucleus which he thought to be the stalk nucleus. From *Microcycas*, then, through *Dacrydium* to *Pinus* we have a reduction series in the spermatogenous tissue. The inequality of the sperms, so common in conifers, is only a further step in this reduction.

The tendency for two body cells to develop in *Dacrydium* may be related to the transverse division of the generative cell. In *Pinus* the inner cell has the advantage at the start, and is thus set apart as the body cell; but in *Dacrydium* both cells begin existence with apparently even chances, being equally free to enlarge. Thus it seems at first a matter of indifference which shall be the body cell and which sterile, and some accident of slightly superior size or better nourishment may be the determining factor.

#### SUMMARY

1. There are two prothallial cells cut off from the main body of the spore. In *Dacrydium Bidwillii* usually only the second divides; in *D. laxifolium* and *D. cupressinum* both divide.
2. The generative cell divides by an anticlinal wall, one daughter cell functioning as a body cell and the other being sterile. In some cases both produce body cells.
3. The walls of the prothallial cells and the two generative daughter cells disappear.
4. The mature pollen grain contains the body cell and five or six free nuclei, according as the first prothallial cell has or has not divided.

This work was carried on under the direction of Professor J. M. COULTER and DR. C. J. CHAMBERLAIN.

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NOTE.—Since this investigation was completed, a paper by JEFFREY and CHRYSLER (The microgametophyte of the Podocarpineae. Amer. Nat. 41: 355-364. 1907) on the same subject has appeared. The paper deals chiefly with *Podocarpus*. The development of the gametophyte corresponds in general with that of *Dacrydium*, but with further divisions of the prothallial cells, resulting sometimes in eight, and an occasional division of the generative cell into three instead of only two cells.

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## EXPLANATION OF PLATE XIX

A Zeiss apochromatic 2<sup>mm</sup> homogeneous immersion objective and compensating ocular 8 were used. All drawings were made with a camera lucida and reduced one-half, giving a magnification when reduced of 960 diameters.

Abbreviations: *t*, tube nucleus; *g*, generative cell; *p<sub>1</sub>*, first prothallial cell; *p<sub>2</sub>*, second prothallial cell; *b*, body cell; *s*, spermatogenous cell; *st*, starch.

*Figs. 1-10, Dacrydium Bidwillii*

FIG. 1. Microspore.  
 FIG. 2. First prothallial cell cut off.  
 FIGS. 3, 4. Cutting-off of second prothallial cell.  
 FIG. 5. Cutting-off of generative cell.  
 FIG. 6. The second prothallial cell divided.  
 FIG. 7. Division of generative cell.  
 FIG. 8. The generative cell divided, the two daughter cells enlarging.  
 FIG. 9. Tube nucleus, body cell, and two prothallial cells; stalk cell not shown.  
 FIG. 10. Walls of stalk and body cells disappearing.  
 FIG. 11. *Dacrydium cupressinum*; both prothallial cells divided.  
 FIGS. 12, 13. *Dacrydium laxifolium*; tube nucleus entering the tube; body cell and prothallial and stalk nuclei in the grain.

